Supplementary Information

Potential impact of organic ligands on the antibacterial activity of silver nanopaticles

Peng Xue[a],Yixuan Shan[b],Qihui shen[a,b],Yingdi Li[a],Jiayu Jiang[a] ,Yan Liu[a,b,*],Xiaoyang Liu[a,*]

[a] State Key Laboratory of Inorganic Synthesis and Preparative Chemistry, College of Chemistry, Jilin University, 2699 Qianjin Street, Changchun 130012 (P. R. China) E-mail: liuxy@jlu.edu.cn

[b] Department of Chemistry and Pharmaceutical Engineering, Jilin Institute of Chemical Technology,45 Chengde Street, Jilin 132022 (P. R. China) E-mail: liuyanjlu@126.com

Experiment

Materials

3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) and Tetraethyl orthosilicate(TEOS,98%), and Melittin were obtained from Sigma,3-Mercaptopropyl trimethoxysilane (MPTMS, 97%) was obtained from J&K Chemical Ltd,AgNO3(99.8%), NaOH (99%), and ethanol (99.8%) were purchased from Shanghai Chemical Reagents Company,2-Mercaptoethanol,2-Phenylethanethiol,1,2-Ethanedithiol,mercaptoacetic acid were purchased from Shanghai Macklin Biochemical Co.,Ltd. Escherichia coli DH5α was purchased from Takara.

Methods

Synthesis of AgNPs@silica dioxide submicrospheres which have different ligands

AgNPs@silica dioxide submicrospheres(AgNPs@TSMs)was prepared by a slightly modified procedure developed by Liu(2017)[26]. Firstly, TEOS(2.3ml), NH_4OH(3ml), and H_2O(1ml) were mixed with 99.9% EtOH (60ml)and the solution was stirred for 6 h at 20 ℃. Subsequently, a EtOH solution (0.5ml) containing MPTMS(0.1ml) was added in and stirred vigorously for 24 h at 20 ℃. The precipitates of SiO_2 submicrospheres were collected by centrifugation at 8000 rpm, followed by washing with EtOH and water three times separately. Second, SiO_2

submicrospheres were added into $AgNO_3$ solution (25ml)with stirring for 10 min. The resulted suspension were centrifugated at 8000 rpm for 5 min and repeated washing three times with water finally,different dosages of ligands solution (2-Mercaptoethanol,2-Phenylethanethiol,1,2-Ethanedithiol,mercaptoacetic acid) were added into AgNPs@SiO_2 suspension, which the molar proportion of ligands solution to AgNPs is 5:1, and the solution was stirred for 5h at room temperature. The resulted AgNPs@SiO_2 with different ligands were washed three times with water and stored in EtOH.

Antimicrobial activity

The antibacterial activity of AgNPs@SiO_2 with different ligands was investigated against Escherichia coli (E. coli) DH5α by two methods: disk diffusion method and the minimal inhibitory concentration (MIC).

Disk diffusion method.

The whole process was performed in clean bench，and the plates and other tools were sterilized by ultraviolet lamp for 15 min. Then lawns of test bacteria (about 1×10^{-6}CFU/mL) were prepared on Tryptone Soya Agar(TSA)plates. Filter paper discs which were immersed in suspension of AgNPs@SiO_2 with different ligands for 5 mins were placed upon the lawns and allowed to dry for 10 min at room temperature, after which they were incubated at 37℃ for 24 h. Then inhibitory action of tested samples on the growth of the bacteria was proved by the existence of inhibition zone.In addition， four vertical radius from the core of filter paper discs to the edge of inhibition zone were measured by vernier caliper owing to the filter paper discs were not perfect circle.The final result was average value of four measurement.

Minimal inhibitory concentration (MIC).

All bacteria were cultured to exponential phase under shaking at 37℃ and diluted to 10^6 CFU/ml roughly. The suspensions were centrifuged, followed by resuspension with potassium phosphate buffer (PB) three times. AgNPs@TSMs with different ligands,which the initial concentration was 256μg/ml, were diluted to double volume every time in 96-well plates,and 100μl sample solution was added for each concentration . Then bacteria suspensions (100μl) were added into each well and

incubated at 37℃ for 2 h. Finally, suspensions of each dilution(100μl) were plated out on luria broth and incubated overnight at 37℃. The experiment were carried out in triplicate to confirm reproducibility.

Kinetics of antibacterial activity

The bacteria were cultured to about 10^6 CFU/ml and then treated with a series of AgNPs at the the maximum value of MIC range. The suspensions were taken out every 10 mins and colony number was recorded by plate counting method.The specific operation is:the bacterial suspensions was diluted to multiple of 10^{-4},10^{-5} and 10^{-6},every dilution gradient was prepared three groups,and 0.2ml of dilution was plated out on luria broth and incubated 12h at 37℃.The Colony-Forming Units(CFU) was calculated according to the following equation:

$$CFU=C\times d\times 5 \qquad (1)$$

Where C is average colony number,d is dilution multiple.

Cytotoxicity tests.

The cytotoxicity of AgNPs@SiO_2 with different ligands was measured by the MTT assay method. The human cerebral microvascular endothelial cells(hCMEC/D3) line was cultured in RPMI-1640(GIBCO,USA) Medium and incubated in a humidified incubator at 37℃ containing 5% CO_2.HCMEC/D3 cells(100μL),were seeded at each well of a 96-well plate, followed by incubation overnight at 37℃.After that,AgNPs@TSMs with different ligands were diluted to double volume every time from 256μg/ml to 1μg/ml,and added to each well and incubated for 12h.Then 20 μL of dimethyl thiazolyl diphenyl (MTT) solution was added and the incubation continued for 3 h at 37 ℃ in a humidified 5% CO_2 -containing atmosphere. Medium was then removed, and 150 ml dimethyl sulfoxide was added. The absorbance of the supernatant at a wavelength of 490 nm was measured.The calculation of cell viability was by the ratio between optical density of 490nm(OD_{490}) of cells incubated with AgNPs and normal cultured.

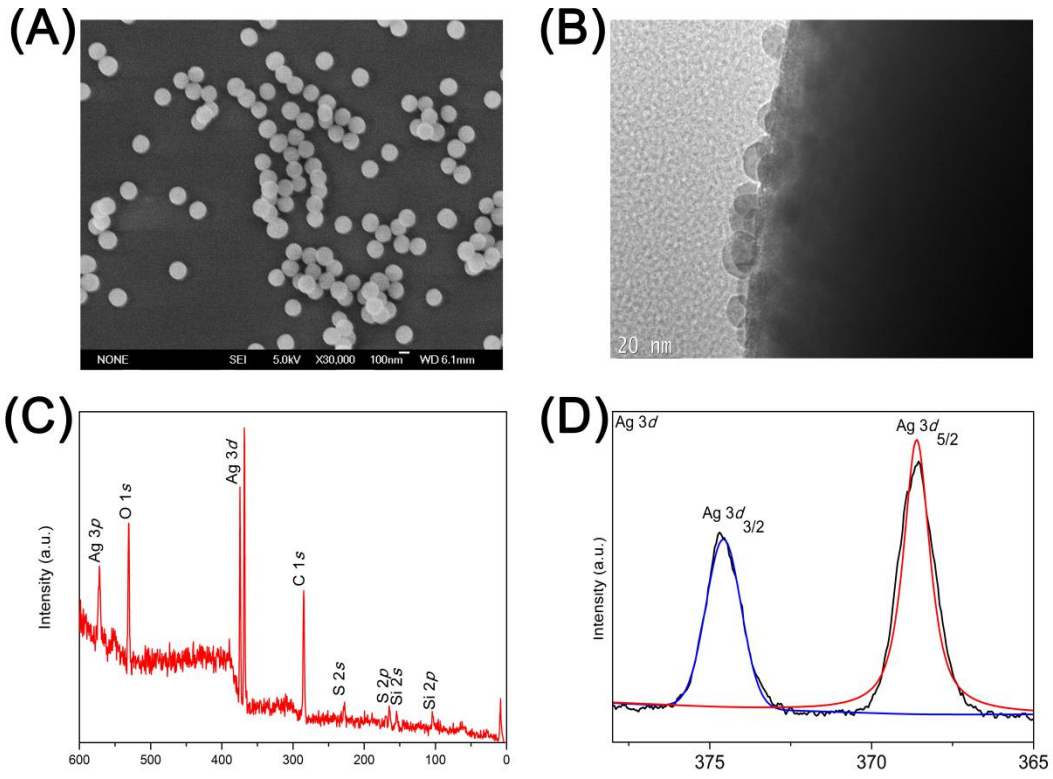

Fig.S1 (A)SEM images of thiol group (RSH) functionalized silicon dioxide submicrospheres(TSMs),(B) HRTEM images of AgNPs on the surface of TSMs,(C) and (D) X-ray photoelectron spectroscopy(XPS) survey spectrum of the AgNPs.